STEREOSELECTIVE SYNTHESIS OF NUCLEOSIDE ANALOGUES

FIELD OF THE INVENTION

The present invention relates generally to a novel method for the preparation of nucleoside analogues and their precursors and more particularly to a method of preparing a nucleoside analogue by the use of specific enzymes to stereoselectively produce dioxolane nucleoside analogues or their precursors.

BACKGROUND OF THE INVENTION

The pharmacological activity of pharmaceutical compounds (drugs) depend mainly on their interaction with biological matrices (drug targets), such as proteins (receptors and enzymes), nucleic acids (DNA and RNA) and biomembranes (phospholipids and glycolipids). All these drug targets have complex three-dimensional structures which are capable of binding specifically to the drug in only one of the many possible arrangements in the three-dimensional space. It is the three-dimensional structure of the drug target that in part, determines which of the potential drug is bound within its cavity and with what affinity.

The spatial arrangement of atoms in an asymetric molecule is termed chirality. Chirality results in the creation of stereoisomers. Stereoisomers are compounds with identical chemical composition and atom connectivity (i.e. same constitution), but different arrangements of the atoms in space (i.e. different configurations). Stereoisomers are classified according to the number of

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chiral centers in each molecule and the spatial arrangement of the chiral center.

Chiral centers of organic molecules include chiral carbon atoms which have four different substituents connected thereto and arranged in a generally tetrahedral configuration. Another type of chiral center is a chiral plane oriented along a rigid C=C bond that has at least two different substituents connected to the remaining four bond positions in that arrangement.

The chirality of molecules that are the subject of the present application refer to chirality created by chiral atoms and not chiral bonds. The following discussions will be limited to chirality created at one or more chiral carbon atoms which have four different substituents bound to each of the four different binding sites of the carbon.

When a molecule has a single chiral carbon, there are two stereoisomers that are mirror images of each other. This pair of isomers is termed enantiomers or an enantiomeric pair. When there are two chiral carbon atoms, there are four stereoisomers and two pairs of mirror images or enantiomers. A stereoisomer which is not a mirror image of another stereoisomer is a diastereoisomer.

One type of stereochemical distinction relates to cyclic sugars or analogues of cyclic sugars. Cyclic sugars can be designated as a particular anomer depending upon the stereochemical configuration.

The term "anomer" designates the spatial arrangement of cyclic sugars or analogues or derivatives thereof that

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have two chiral centers in a five or six member ring.
The anomeric designation defines the relative
configuration of the two chiral centers relative to a
hypothetical plane defined by the ring. The chiral
centers typically have two substituents outside the ring.
One substituent is a H. The other substituent is a
larger moiety such as a hydroxyl, methoxyl, purine or
pyrimidine base, carboxyl, etc.

When the two larger constituents on each chiral center
are on the same side of the plane in the ring, they are
defined as a β-anomer (cis-isomer). When two larger
moieties are on opposite sides of the plane in the ring,
they are defined as the α-anomer (trans-isomer). An
anomer is a type of diastereoisomer.

Because chirality may affect biological activity or
toxicity, it is important from the point of view of drug
development to evaluate the physiological effect of each
isomer. Frequently, one stereoisomer is considerably
more active than the other. In other situations, the
non-active isomer may inhibit the activity of the more
active form. In some instances, the less preferred
stereoisomer may be equally potent but have greater
toxicity than the preferred stereoisomer. In each of
these instances, the therapeutic effect of a drug can be
increased if the single most preferred stereoisomer is
administered in higher purity.

The current trend in the drug markets reflects a greater
use of single stereoisomer drugs. The sales of single
stereoisomer drugs have increased considerably. In 1995,
sales of single stereoisomer drugs reached $61 billion

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worldwide. In the year 2000, the annual worldwide sales
are expected to reach $90 billion.

An important class of pharmacological agents relate to
3'-oxa-substituted 2',3'-dideoxynucleoside analogues
("dioxolane nucleoside analogues"). These compounds have
two chiral centers corresponding to the substituted
carbons 2 and 4 of the dioxolane ring (C2 and C4
respectively). Thus each compound can exist as four
different stereoisomers depending on the position of both
substituents with respect to the dioxolane ring.

The stereoisomers of a dioxolane nucleoside analogue are
represented by the following diagrams where the letter B
represents a purine or pyrimidine base or an analogue or
derivative of a purine or pyrimidine base as defined
herewith.
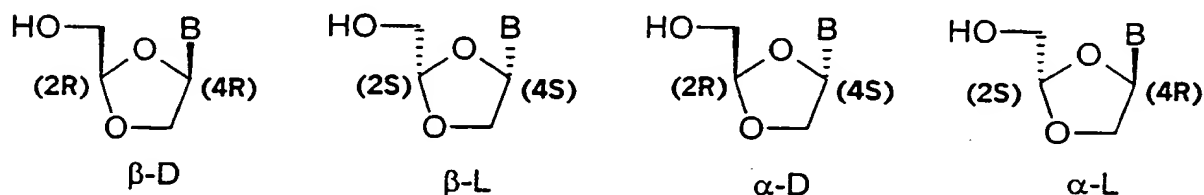
For the purpose of consistency, the same stereochemical
designation is used when the methyloxyl moiety or the
base moiety is replaced with another substituent group.

The C2 carbon in each of the above formula is the carbon
atom in the ring that connects the methyloxy group to the
dioxolane ring. The C4 carbon is the carbon atom in each
of the above formula that connects the base (B)
substituent to the dioxolane ring.

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The four stereoisomers represented above correspond to two pairs of enantiomers. The β-anomers represent one set of enantiomers, the β-L enantiomer and the β-D enantiomer. The α-anomers represent the other set of enantiomers, the α-L enantiomer and the α-D enantiomer.

Compounds with D-configuration have an outward directed methyloxy group when the ring is oriented in the plane of the page with the oxygen in the first position (O3) at the top of the page with the carbon in the two position (C2) on the left side as illustrated above. This is also represented by the designation (2R). Compounds having an L-configuration has inward directed methyloxy group when the ring is oriented in the plane of the page with the O3 oxygen at the top of the page with the C2 carbon on the left side as illustrated above. This is also represented by the designation (2S).

A variety of dioxolane nucleoside analogues have been identified to have antiviral and anticancer activity. For example, 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-2-aminopurine (β-D-DAPD) and its metabolite 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-guanine (β-D-DXG) have been reported to have potent and selective activity against human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Rajagopalan et al., Antiviral Chem. Chemother.,1996, 7(2), 65-70) Similarly, 1-(β-L-2-hydroxymethyl-1,3-dioxolan-4-yl)-thymine (Dioxolane-T)(Norbeck et al., Tetrahedron Lett., 1989,30, 6263-66) possess anti-HIV and anti-HBV activity. 1-(β-L-2-hydroxymethyl-1,3-dioxolan-4-yl)-cytidine (β-L-OddC) (Bednarski et al., Bioorg. Med. Chem. Lett., 1994, 4,

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2667-72) was discovered to have potent anti-tumor activity towards human prostate as well as renal carcinoma (Kadhim et al., Can. Cancer Res., 57(21),4803-10, 1997). β-L-OddC is the first nucleoside analogue with an L-configuration shown to have anticancer activity. Since stereoisomers of dioxolane nucleosides usually have different biological activities and toxicity, obtaining the pure therapeutically active isomer becomes crucial.

Chiral synthetic methods have improved over the past several years with respect to synthetic techniques that result in single stereoisomer compounds. However, there is a present need to find novel synthetic methods which can be widely used to form a particular stereoisomer with greater efficiency and purity.

For example, for many years a person of ordinary skill in the art could use enzymes to separate enantiomers of dioxolane compounds. However, it is not known in the art how to produce a dioxolane nucleoside analogue using a step of separating an anomeric mixture of certain dioxolane precursors using enzymes to produce a stereochemically pure end product with greater efficiency and purity.

Because stereochemically pure dioxolane nucleosides are an important class of compounds due to their known antiviral and anticancer activity, there is a need for other inexpensive and efficient stereoselective methods for their preparation. The present invention satisfies this and other needs.

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SUMMARY OF THE INVENTION

The present invention provides a novel process for making dioxolane nucleoside analogues with a high degree of steric purity, greater efficiency and higher yields.

Specifically, the present invention provides a process for making dioxolane nucleoside analogues with a high degree of steric purity which includes the use of certain hydrolytic enzymes for separating β and α anomers from an anomeric mixture represented by the following formula A or formula B:

(A)   (B)
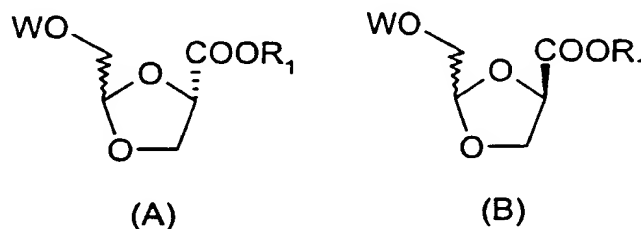
In the above formula, W is benzyl or benzoyl; R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl.

The process involves the step of hydrolyzing the mixture of compounds represented by formula A and formula B with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, proteinase from *Bacilius licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species

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type B, lipase from *Pseudomonas cepacia* and bacterial proteinase. The process stereoselectively hydrolyses predominantly one anomer to form a product where R_1 of formula A and formula B is replaced with H. The other anomer remains substantially unhydrolysed. The process also comprises separating the hydrolyzed product from unhydrolysed starting material.

The process of one embodiment further includes the step of stereoselectively replacing the functional group at the C4 position of the dioxolane (e.g. $COOR_1$) with a purinyl, pyrimidinyl or analogue or derivative thereof to produce a dioxolane nucleoside analogue that has a high degree of steric purity.

According to one embodiment of the invention, the step of hydrolyzing results in a starting material having an anomeric purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 95%. In an additional embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 98%.

According to one embodiment of the invention, the step of hydrolyzing results in a product having an anomeric purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 95%. In an

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additional embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 98%.

In one embodiment of the present invention, W of formula A or formula B is benzyl and the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*. In another embodiment, the enzyme is α-chymotrypsin. In yet another embodiment, the enzyme is bovine pancreatic protease.

In one embodiment of the present invention, W of formula A and formula B is benzoyl and the enzyme is selected from the group consisting of protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, bacterial proteinase, lipase from *Pseudomonas cepacia*. In another embodiment, the enzyme is selected from the group consisting of *Aspergillus oryzae protease*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease from *Streptomyces griseus*, pronase protease from *Streptomyces griseus*, and lipase from *Rhizopus arrhizus*. In yet another embodiment, the enzyme is selected from the group comprising *Aspergillus oryzae* and proteinase from *Bacillus licheniformis*.

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In one embodiment, the β-anomer is the predominant product. In another embodiment, the α-anomer is the predominant product. In yet another embodiment, the β-L-enantiomer is the predominant product. In an additional embodiment, the β-D-enantiomer is the predominant product. In yet another embodiment, the α-L-enantiomer is the predominant product. In an additional embodiment, the α-D-enantiomer is the predominant product.

In one embodiment, the invention is a process for stereoselectively preparing a dioxolane nucleoside analogue by separating β and α-anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments. The process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a purinyl or pyrimidinyl or analogue or derivative selected from the group consisting of:
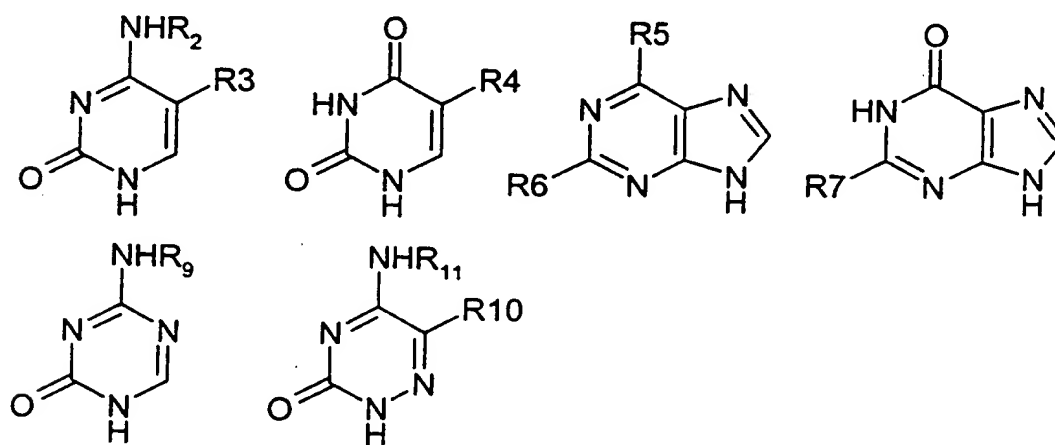
In this embodiment, R_2, R_9 and R_{11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6}

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alkyl. Additionally, R_3, R_4 and R_{10} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino. The process results in the production of a stereochemical isomer of the dioxolane nucleoside analogue.
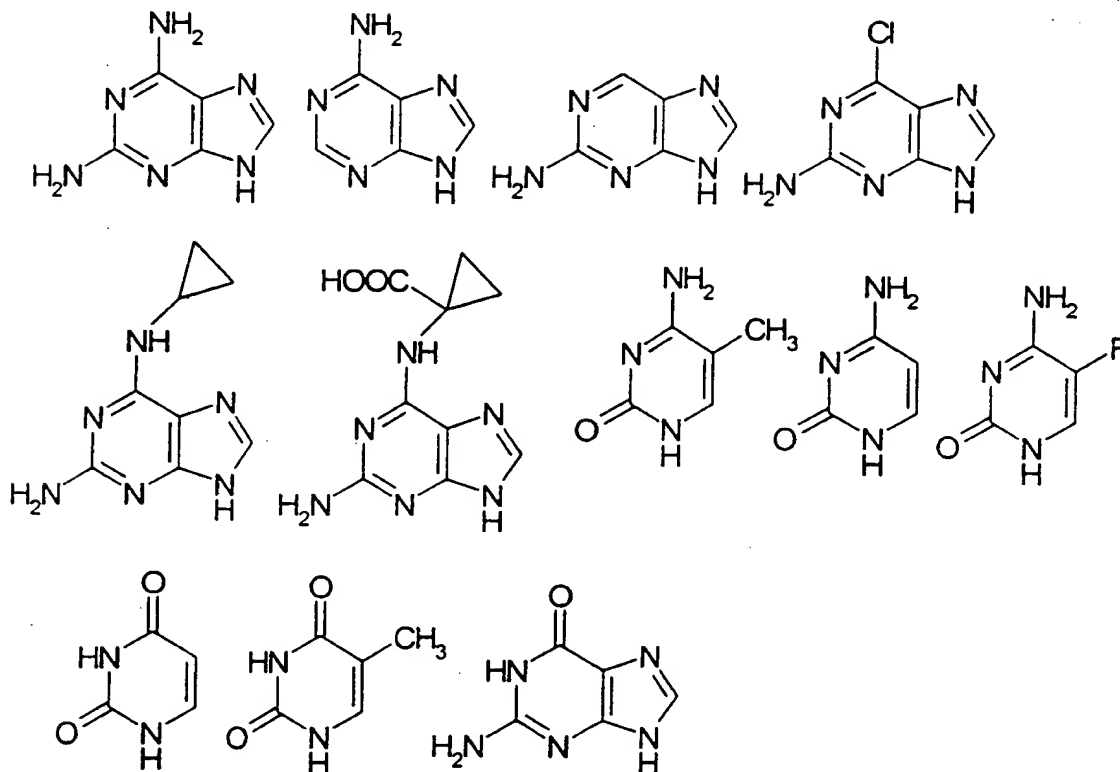
According to one embodiment, the process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a purinyl or pyrimidinyl or derivative selected from the group consisting of:
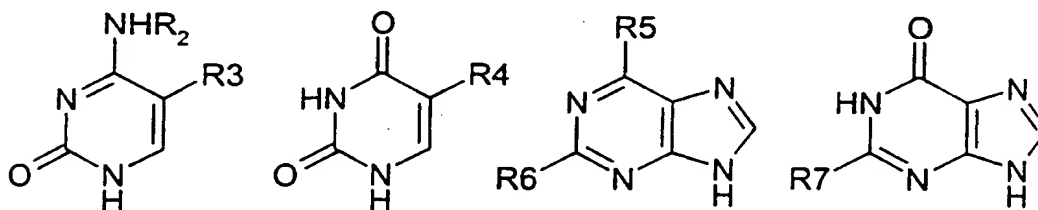
In this embodiment, R_2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl. Additionally, R_3 and R_4 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino. The process results in the production of a stereochemical isomer of a dioxolane nucleoside analogue.

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In another embodiment, the process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a pyrimidinyl or analogue or derivative selected from the group consisting of:
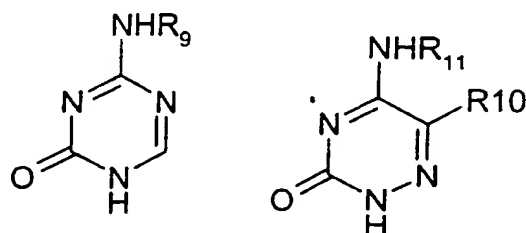
In this embodiment, R_9 and R_{11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$. Additionally, R_{10} is selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3. The process results in the production of a stereochemical isomer of a dioxolane nucleoside analogue.

In another embodiment, the process comprises stereoselectively preparing a dioxolane nucleoside analogue by separating β and α anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments and further comprises stereoselectively replacing the functional group at the

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C4 position (COOR₁) with a moiety selected from the group consisting of:

In another embodiment of the present invention, the process comprises making a dioxolane nucleoside analogue by separating a compound according to formula A or formula B. According to this embodiment, the process includes stereoselectively replacing the R group with a purinyl or pyrimidinyl moiety or analogue or derivative thereof by acylating the second mixture to produce an acylated second mixture. This embodiment also includes the step of glycosylating the acetylated second mixture with a purine or pyrimidine base or analogue or derivative thereof and a Lewis Acid to produce a dioxolane nucleoside analogue.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves a high yield process of separating β and α anomers from an anomeric mixture of dioxolane nucleoside analogue precursors which provides higher yield and greater efficiency. In one embodiment, this method is used in the production of dioxolane nucleoside analogues having a high degree of anomeric purity at lower cost. Additionally, another aspect of the present invention involves synthesizing starting material having a higher degree of anomeric purity.

The present invention provides a process of preparing dioxolane nucleoside analogues having a predominant β-L- configuration using enzymes, namely hydrolases. The procedure improves overall yield and has relatively few steps, thereby improving overall efficiency. The process involves the following steps.

A mixture of anomers represented by formula A or formula B is obtained as described herein in Scheme 1.

(A)   (B)
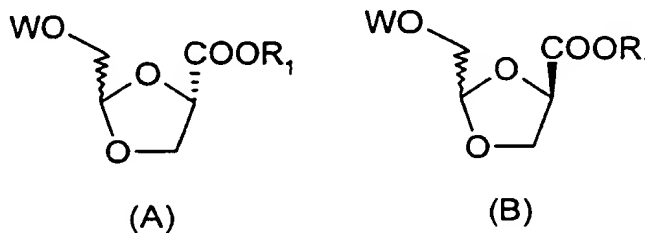
In the above formula, W is benzyl or benzoyl, and R_1 is selected from the group consisting of H, C_{1-6} alkyl and C_{6-15} aryl. The mixture is hydrolyzed with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine

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pancreatic protease, α-chymotrypsin, protease from
Streptomyces caespitosis, substilisin from *Bacillus
licheniformis*, protease from *Aspergillus oryzae*,
proteinase from *Bacillus licheniformis*, protease *from
Streptomyces griseus*, acylase from *Aspergillus melleus*,
proteinase from *Bacillus subtilis*, ESL-001-05, pronase
protease from *Streptomyces griseus*, lipase from *Rhizopus
arrhizus*, lipoprotein lipase from *Pseudomonas* species
type B, lipase from *Pseudomonas cepacia* and bacterial
proteinase. The hydrolyzing step stereoselectively
hydrolyzes the α-anomer of the mixture of either formula
A or formula B. The result is an unhydrolysed β-anomer.
The α-anomer can be separated easily from the β-anomer.
If an anomeric mixture of the compound of formula A is
selected, the result is the production of the compound of
formula C and formula D:
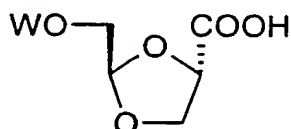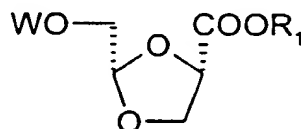
(C)   (D)

If an anomeric mixture of the compound of formula B is
selected, the result is the production of the compound of
formula E and formula F:
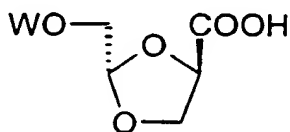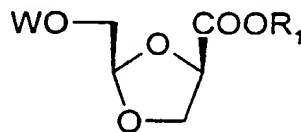

(E)   (F)

The mixture (C)/(D) or (E)/(F) is then subjected to
oxidative decarboxylation which replaces the R_1 group with
an acyl moiety. It is then glycosylated with a purine or
pyrimidine base or analogue or derivative thereof in the

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presence of a Lewis Acid. The final step produces a
dioxolane nucleoside analogue in the β-L configuration
for the mixture (C)/(D) and a dioxolane nucleoside
analogue in the β-D configuration for the mixture
(E)/(F).

At the outset, the following definitions have been
provided as reference. Except as specifically stated
otherwise, the definitions below shall determine the
meaning throughout the specification.

"Nucleoside" is defined as any compound which consists of
a purine or pyrimidine base, linked to a pentose sugar.

"Dioxolane nucleoside analogue" is defined as any
compound containing a dioxolane ring as defined
hereinafter linked to a purine or pyrimidine base or
analogue or derivative thereof. A "dioxolane ring" is
any substituted or unsubstituted five member monocyclic
ring that has an oxygen in the 1 and 3 positions of the
ring as illustrated below:

**Dioxolane
Ring**
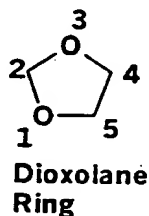
"Purine or pyrimidine base" is defined as the naturally
occurring purine or pyrimidine bases adenine, guanine,
cytosine, thymine and uracil. A purine or pyrimidine that
is a moiety is a purinyl or pyrimidinyl, respectively.

"Alkyl" is defined as a substituted or unsubstituted,
saturated or unsaturated, straight chain, branched chain
or carbocyclic moiety, wherein the straight chain,

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branched chain or carbocyclic moiety can be optionally
interrupted by one or more heteroatoms (such as oxygen,
nitrogen or sulfur). A substituted alkyl is substituted
with a halogen (F, Cl, Br, I), hydroxyl, amino or C_{6-20}
aryl.

"Aryl" is defined as a carbocyclic moiety which can be
optionally substituted or interrupted by one heteroatom
(such as oxygen, nitrogen or sulfur) and containing at
least one benzenoid-type ring (such as phenyl and
naphthyl).

"Carbocyclic moiety" is defined as a substituted or
unsubstituted, saturated or unsaturated, C_{3-6} cycloalkyl
wherein a substituted cycloalkyl is substituted with a
C_{1-6} alkyl, halogen (i.e. F. Cl, Br, I), amino, carbonyl
(i.e. COOH), or NO_2.

A "derivative" of a purine or pyrimidine base refers to
one of the following structures:
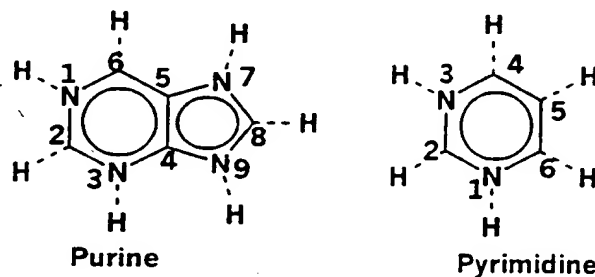
Purine   Pyrimidine

wherein one or more of the pyrimidine H are substituted
with substituents that are known in the art. In the
above illustration, the bonds represented by a broken
line are optional and are present only in cases which
require the bond to complete the valence of the ring
atom. Substitutents bound to the ring members by a

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single bond include but are not limited to halogen such as F, Cl, Br, I; an akyl such as lower akyls; aryl; cyano carbamoyl; amino including primary, secondary and tertiary amino; and hydroxyl groups. Substituents bound to the carbon ring atoms by a double bond include but are not limited to a =O to form a carbonyl moiety in the ring. It is understood that when the ring is aromatic, some of the substitutions may form tautomers. The definition shall include such tautomers.

"Analogue" of a purine or pyrimidine base refers to any derivative of purine or pyrimidine bases that is further modified by substituting one or more carbon in the ring structure with a nitrogen.

"Stereoselective enzymes" are defined as enzymes which participate as catalysts in reactions that selectively yield one specific stereoisomer over other stereoisomers.

"Anomeric purity" is defined as the amount of a particular anomer of a compound divided by the total amount of all anomers of that compound present in the mixture multiplied by 100%.

"Alkoxy" is defined as an alkyl group, wherein the alkyl group is covalently bonded to an adjacent element through an oxygen atom (such as methoxy and ethoxy).

"Alkoxycarbonyl", is defined as an alkoxy group attached to the adjacent group of a carbonyl.

"Acyl" is defined as a radical derived from a carboxylic acid, substituted (by a halogen, C_{6-20} aryl or C_{1-6} alkyl) or unsubstituted by replacement of the -OH group. Like

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the acid to which it is related, an acyl radical may be
aliphatic or aromatic, substituted (by halogen, C_{1-6}
alkoxyalkyl, nitro or O_2) or unsubstituted, and whatever
the structure of the rest of the molecule may be, the
properties of the functional group remain essentially the
same (such as acetyl, propionyl, isobutanoyl, pivaloyl,
hexanoyl, trifluoroacetyl, chloroacetyl and
cyclohexanoyl).

"Alkoxyalkyl" is defined as an alkoxy group attached to
the adjacent group by an alkyl group (such as
methoxymethyl).

"Acyloxy" is defined as an acyl group attached to the
adjacent group by an oxygen atom.

"Oxo" is defined as a =O substituent bonded to a carbon
atom.

As noted above, one embodiment of the present invention
is a process for separating β and α anomers from an
anomeric mixture represented by the following formula A
or formula B:

(A)   (B)
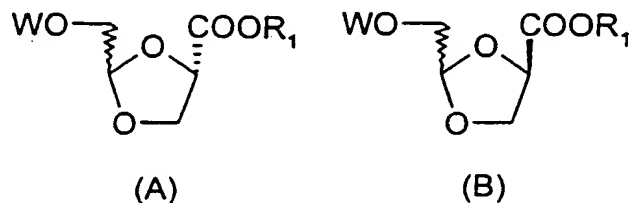
wherein W is benzyl or benzoyl and R_1 is selected from the
group consisting of C_{1-6} alkyl and C_{6-15} aryl.

In one embodiment, the process stereoselectively
hydrolyses predominantly the α-anomer to form a product

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where R₁ of formula A and formula B is replaced with H.
The β-anomer remains substantially unhydrolyzed. The
process also comprises separating the hydrolyzed product
from unhydrolyzed starting material.

The process of making a β-L dioxolane nucleoside analogue
begins with the preparation of starting materials.
Scheme 1 depicts the manufacture of a mixture that
includes formula A or B.

Scheme 1
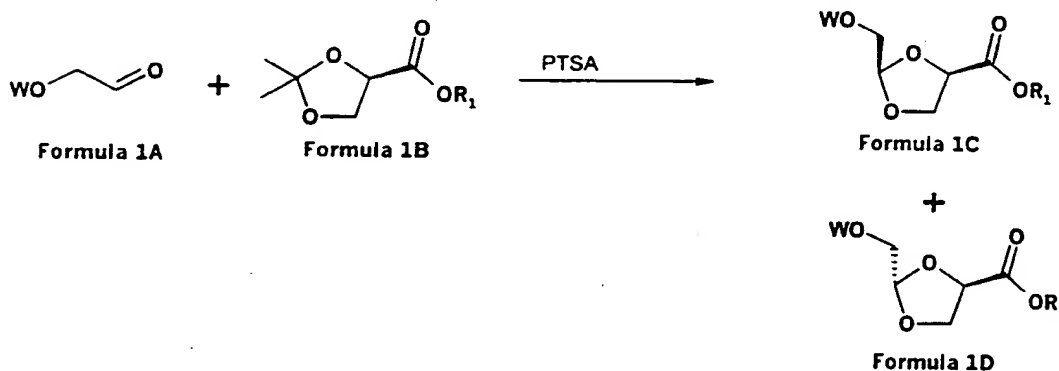
An oxyacetaldehyde represented by formula 1A (wherein W
is benzyl or benzoyl) is reacted with 1,3-dioxolane-4-
(4R)carboxylic acid-2,2-dimethyl-methyl ester (formula
1B) in approximately equimolar proportions. The
dioxolane of formula 1B has a chiral center at the C4
carbon. The reaction occurs in a toluene solvent. The
mixture is heated to 58°C. The catalyst, PTSA, is added.
The mixture is heated to a temperature between 64-67°C. A
vacuum is applied at 70 kPa, and the reaction proceeds
for 40 minutes. Traces of solvent are then removed by
high vacuum. The catalyst is removed by filtration using
a 1:1 ratio of Hexane:EtOAc as an eluent. In one
embodiment, the preferred filter is a silica gel pad.

The resulting product is a crude oil containing a mixture of the compounds of formula 1C and 1D, wherein the ratio is 2:1 of (1C:1D) respectively.

It can be appreciated by a person of skill in the art that the reaction conditions can be adjusted to optimize the purity of the stereoisomers. In one embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done in the presence of catalyst in an amount between about 1.0 wt% and 10.0 wt% of the starting material. In another embodiment the amount of catalyst is between about 2.5 wt% and about 5.5 wt% of the starting materials. In yet another embodiment, the amount of catalyst is between about 3.0 wt% and about 5.0 wt%. In still another embodiment, the amount of catalyst is between about 3.5%. and about 5.5%. In another embodiment, the amount of catalyst is between about 2.5 wt% and about 7.5 wt%. In another embodiment the amount of catalyst is about 5.0 wt%.

In an embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done at a temperature ranging from about 40°C to about 80°C. In another embodiment of the present invention, the temperature ranges from about 50°C to about 75°C. In still another embodiment, the temperature ranges from about 60°C to about 70°C. In an additional embodiment, the temperature ranges from about 65°C to about 79°C.

In an embodiment of the present invention, the reaction time between the compound of formula 1A and the compound of formula 1B corresponds to a period ranging from about

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30 minutes to about 2 hours. In yet another embodiment, the period ranges from about 30 minutes to about 1 hour. In still another embodiment, the period ranges from about 30 minutes to about 50 minutes.

It will be appreciated by a person of ordinary skill in the art that the C4 carbon is chiral. Because this carbon is not involved in the reaction, the chirality is preserved at that carbon. A starting material can be selected to have a (4S) or (4R) stereochemistry.

According to one embodiment, it is preferable that the resulting product is an anomeric mixture favoring the β-L configuration over the α-L configuration. To achieve such a result, the starting material represented by formula 1B (4S) is selected and shown below:

Formula 1B (4S)
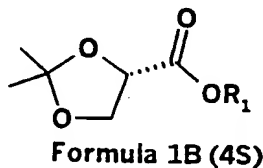
The reaction proceeds according to the principles described above. The resulting product, according to one embodiment, will have an anomeric purity of the β-L anomer over the α-L anomer of greater than 55%, preferably 60% and more preferably 65%.

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According to one embodiment, the present invention is a
method of separating β-anomers from α-anomers according
to the following Scheme 2:

Scheme 2
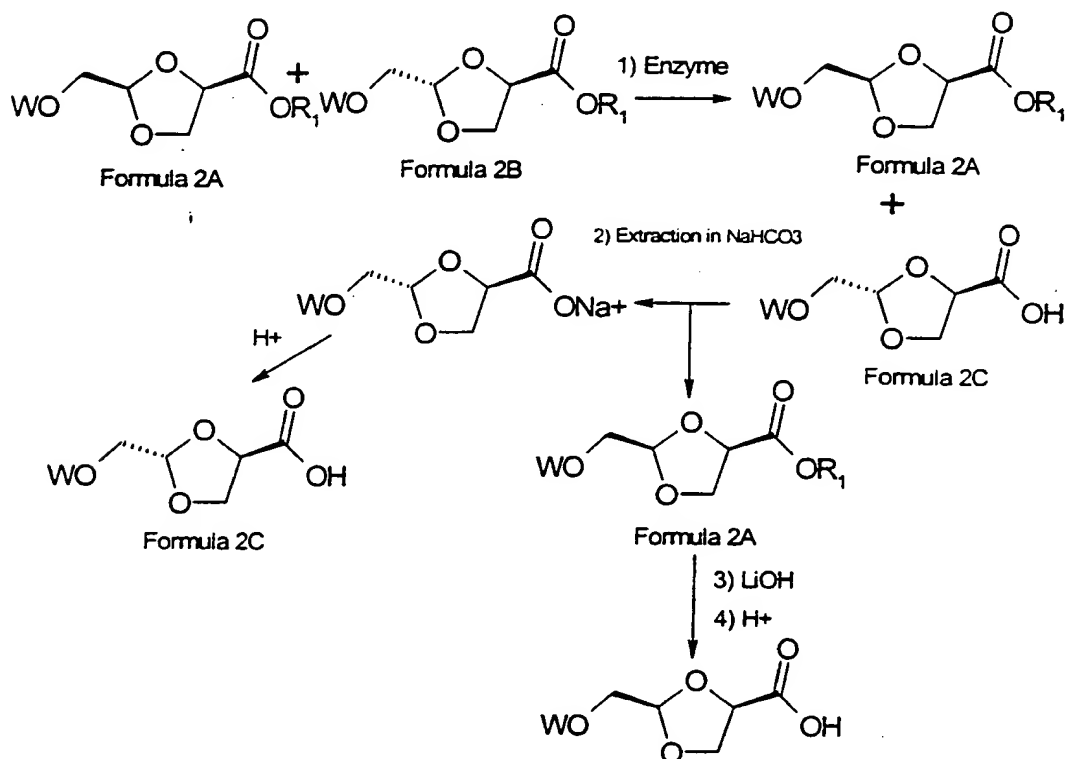
According to one embodiment, a mixture of anomers is
obtained as represented by formula 2A or formula 2B. A
mixture represented by formula 2A or formula 2B can be
obtained according to the reaction described above or
according to any method known in the art.

The reaction is prepared as follows: A portion of the
material represented by formula 2A and formula 2B is
weighed into a reaction vessel. For a small scale
reaction, about 0.001 mmol of the mixture of formula 2A
and formula 2B is added to about 46 mL of a 5 mmol
solution of BES buffer (for a final concentration of

about 2 mM in substrate). For a preparative scale
reaction, about 0.8 mmol of the mixture is added to about
10 mL of buffer (for a final concentration of about 80 mM
in substrate). The pH of the buffer, according to one
embodiment, should be between 7.0 and 7.5 and preferably
7.2.

The enzyme is selected from the group consisting of
cholesterol esterase, ESL-001-02, horse liver esterase,
bovine pancreatic protease, α-chymotrypsin, protease from
Streptomyces caespitosis, substilisin from *Bacillus
licheniformis*, protease from *Aspergillus oryzae*,
proteinase from *Bacillus licheniformis*, protease from
Streptomyces griseus, acylase from *Aspergillus melleus*,
proteinase from *Bacillus subtilis*, ESL-001-5, pronase
protease from *Streptomyces* griseus, lipase from *Rhizopus
arrhizus*, lipoprotein lipase from *Pseudomonas* species
type B, lipase from *Pseudomonas cepacia* and bacterial
proteinase.

The commercial sources of the enzymes are readily
available to a person of ordinary skill in the art.
Particularly, some of the materials can be obtained from
the following sources: Bovine cholesterol esterase was
purchased from Genzyme (Cambridge, MA); ESL-001-02 from
Diversa Corp. (San Diego, CA); Horse liver esterase and
subtilisin from Bacillus licheniformis from Fluka Chemie
(Oakville, ON); Bovine pancreas protease type 1, α-
chymotrypsin and *Streptomyces caespitosis* from Sigma-
Aldrich (Oakville, ON).

In another embodiment, the enzyme is selected from the
group consisting of protease from *Aspergillus oryzae*,

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proteinase from *Bacillus licheniformis*, subtilisin from
Bacillus licheniformis, protease *from Streptomyces
griseus*, acylase from *Aspergillus melleus*, proteinase
from *Bacillus subtilis*, ESL-001-005, pronase protease
from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*,
lipoprotein lipase from *Pseudomonas* species type B,
bacterial proteinase and lipase from *Pseudomonas cepacia*.
The selection of one of these enzymes is preferred
according to this embodiment when the oxyacetaldehyde
represented by the compound of formula 1A in Scheme 1 is
selected to be benzoyloxyacetaldehyde.

According to another embodiment of the invention, the
oxyacetaldehyde represented by the compound of formula 1A
is benzoyloxyacetaldehyde. According to this embodiment,
the enzyme is selected from the group consisting of
protease from *Aspergillus oryzae*, proteinase from
Bacillus licheniformis, subtilisin from *Bacillus
licheniformis*, pronase protease from *Streptomyces
griseus*, and lipase from *Rhizopus arrhizus*. In yet
another embodiment, the enzyme is selected from the group
consisting of protease from *Aspergillus oryzae* and
proteinase from *Bacillus licheniformis*.

In yet another embodiment, the enzyme is selected from
the group consisting of cholesterol esterase, ESL-001-02,
horse liver esterase, bovine pancreatic protease, α-
chymotrypsin, protease from *Streptomyces caespitosis* and
substilisin from *Bacillus licheniformis*. The selection
of one of these enzymes is preferred according to this
embodiment when the oxyacetaldehyde represented by the
compound of formula 1A is benzyloxyacetaldehyde.

The stereospecific enzyme selected is then added to begin

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the hydrolysis reaction. The enzymatic reaction
hydrolyzes primarily the α-anomer by replacing the R_1
group of the α-anomer of the compound of formula 2B with
H to form the compound of formula 2C. The amount of the
enzyme added can be determined according to principles
known by any person of ordinary skill in the art.
According to another embodiment, about 500 μL was added
to begin the reaction. The rate and degree of hydrolysis
was monitored by a pH-stat according to principles known
in the art. As the compound of formula 2B is hydrolyzed,
the pH of the mixture decreases. Thus, the change in pH
as monitored by a pH stat corresponds to the completeness
of the reaction.

If the reaction time is allowed to proceed longer than
the optimal reaction time, the β-anomer may be converted
resulting in lower anomeric purity of the final product.
If the reaction time is too short, less than optimal
amount of the α-anomer is converted resulting in a lower
anomeric purity of the remaining unhydrolyzed reactant.
According to one embodiment, the reaction is allowed to
proceed until 43% completion. It will be appreciated by
a person of ordinary skill in the art that the exact
degree of completion may change depending upon the
reactant used, the enzyme used and other principles known
to a person of ordinary skill in the art.

As noted, the ester starting material is separated from
the hydrolyzed acid product when the desired endpoint is
reached. The ester starting material and the hydrolysed
product are separated by increasing the pH of the
solution to more than pH 7.0 with bicarbonate solution
and extracting with ethyl acetate (for example, 3 x 20

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mL). The unhydrolyzed starting material is extracted in the ethyl acetate and the hydrolyzed product remains in salt form in the aqueous solution. The pH of the solution is then adjusted to pH 2. The hydrolyzed product is further extracted with ethyl acetate (for example, 3 x 20 mL). The reactants and the products are dried with $MgSO_4$, filtered and concentrated *in-vacuo*.

Alternatively, the unhydrolyzed product can be hydrolyzed by procedures known in the art such as reaction with LiOH followed by acidification.

Because of the enzyme selectivity, the anomeric purity of the hydrolyzed and separated β-anomer is considerable. Furthermore, the anomeric purity of the unhydrolyzed and separated α-anomer is also considerable. In one embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 80%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 90%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 95%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 98%.

In another embodiment, the procedure of Scheme 2 is followed except the anomeric mixture represented by formula 2A or 2B is replaced with an anomeric mixture represented by formula 2D and 2E, respectively.
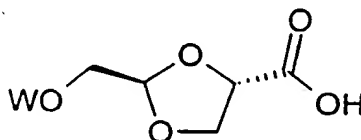
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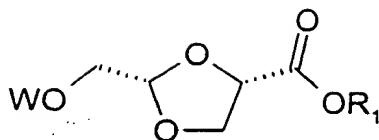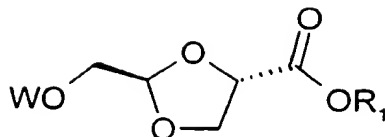
Formula 2D   Formula 2E
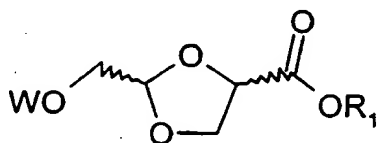
According to this embodiment the α-anomer represented by
formula 2E is hydrolyzed. The result is the separation of
the hydrolyzed α-anomer represented by formula 2F from
the unhydrolyzed β-anomer represented by formula 2D.

Formula 2F

In another embodiment, the procedure of Scheme 2 is
followed except a mixture represented by formula 2A and
2B is replaced with a mixture of four stereoisomers
represented by formula 2G.

Formula 2G

According to this embodiment, the α-anomer containing
both D and L enantiomers is hydrolyzed. The result is
the separation of the hydrolyzed α-anomer containing both
D and L enantiomers from the unhydrolyzed β-anomer
containing both D and L enantiomers.

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After hydrolysis, purification and oxidative decarboxylation, the resulting dioxolane ring can be linked with a purine or pyrimidine base or analogue or derivative. There are several examples known by skilled artisan on how to link a purine or pyrimidine base or analogue or derivative to the dioxolane ring. For example, PCT Publ. No. WO/97/21706 by Mansour et al. describes one method of stereoselectively attaching the purine or pyrimidine base or analogue or derivative to a dioxolane ring. WO/97/21706 is incorporated herein fully by reference.

According to the process disclosed in WO/97/21706 the starting material is an acylated dioxolane ring. The starting material of the procedure disclosed in WO/97/21706 can be obtained by oxidative decarboxylation of a product of Scheme 2 discussed above. Oxidative decarboxylation destroys the stereochemistry of the C4 carbon while preserving the stereochemistry of the C2 carbon.

As noted, the oxidative decarboxylation step occurs after the hydrolysis step of Scheme 2. A compound having the desired stereochemistry on the C2 carbon is selected. For each mmol of compound that is processed, it is dissolved in between about 2.5 and about 4.0 mL of acetonitrile. In another embodiment, between about 3.0 and about 3.5 mL of acetonitrile was added for each mmol of compound. In yet another embodiment, between about 3.3 and about 3.4 mL of acetonitrile was added for each mmol of compound.

For each mmol of compound, between about 0.08 and about 0.12 mL of pyridine was added. In another embodiment,

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between about 0.09 and about 0.11 mL of pyridine was added for each mmol of compound. In yet another embodiment, approximately 0.1 mL of pyridine was added for each mmol of compound.

To this mixture, between 1.1 and 1.5 mmoles of $Pb(OAc)_4$ was added for each mmol of compound. In another embodiment, between about 1.2 mmoles and about 1.4 mmoles of $Pb(OAc)_4$ is added for each mmol of compound. In yet another embodiment, about 1.3 mmoles of $Pb(OAc)_4$ is added for each mmol of compound.

Thereafter, the mixture was stirred for 18 hours at room temperature. Then, the mixture was poured into a saturated solution of $NaHCO_3$. Between approximately 2.0 and 3.0 mL of $NaHCO_3$ were used for each mmol of compound. In one embodiment, between about 2.5 mL and about 2.7 mL, and more preferably about 2.6 mL of $NaHCO_3$ was used for each mmol of compound. The solution was then stirred for an additional 30 minutes. The organic layer was separated from the aqueous layer by four extractions of ethyl acetate. Extracts were combined, dried on anhydrous Na_2SO_4 and evaporated under a vacuum. Optionally, the crude can be further purified by chromatography on silica gel using a gradient of 0-15% ethyl acetate in hexane.

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In one embodiment of the present invention, the oxidative decarboxylation step is followed by glycosylation. The glycosylation is represented by the following Scheme 3.

SCHEME 3

Formula 3A

Formula 3B

Formula 3C

Formula 3D
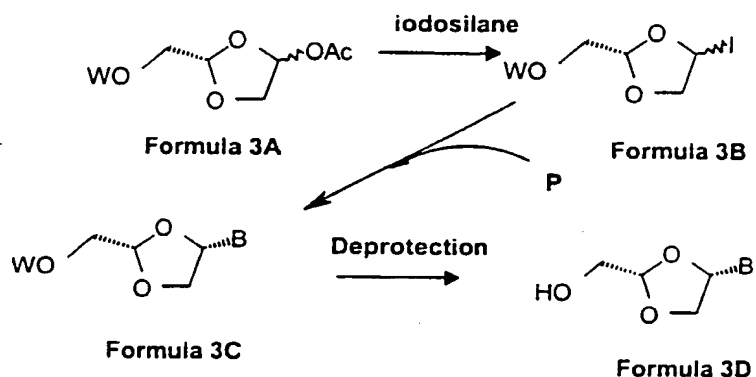
The first step in the glycosylation procedure is to obtain a compound with the desired stereospecificity at the C2 carbon. According to one embodiment, a compound having an S stereochemistry at the C2 carbon, as represented by the compound of formula 3A is preferred. The result is that a higher ratio of the β-L anomer is in the product 3C. According to another embodiment, compound having an R stereochemistry at the C2 carbon is preferred. The result is a product that has a higher ratio of the β-D anomer in the final product.

The compound of formula 3A is reacted with an iodosilane to produce the compound of formula 3B. In one embodiment, the iodosilane is iodotrimethylsilane.

In another embodiment, the iodosilane is diiodosilane. Important to the reaction is that it occurs at low temperatures. According to one embodiment, the temperature is preferably between 0 °C and -78°C prior to

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glycosylation with silylated pyrimidine or purine base or analogue or derivative thereof.

The iodo intermediate represented by formula 3B is then dissolved in dichloromethane and is cooled down to between 0° C and -78° C. A purine or pyrimidine base or analogue or derivative thereof is then selected. According to one embodiment, the purine or pyrimidine base or analogue or derivative thereof is selected from the following group:
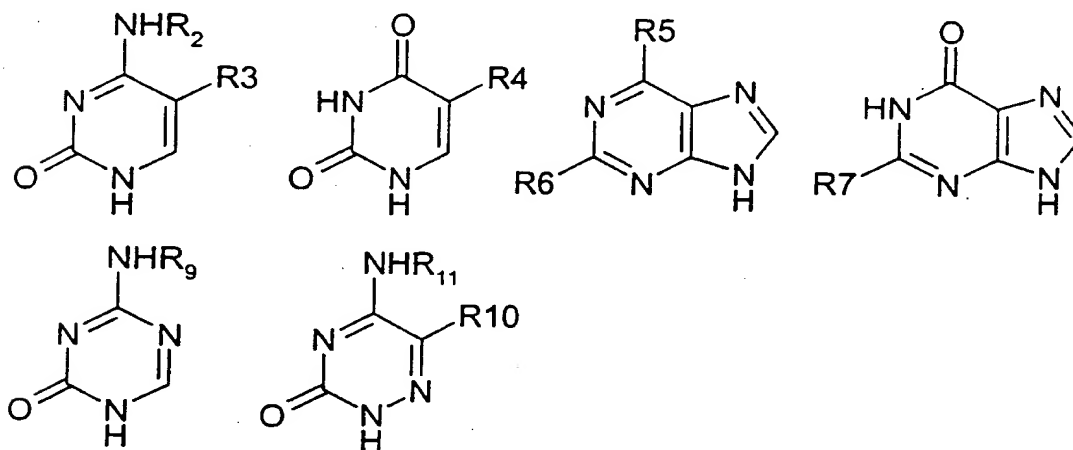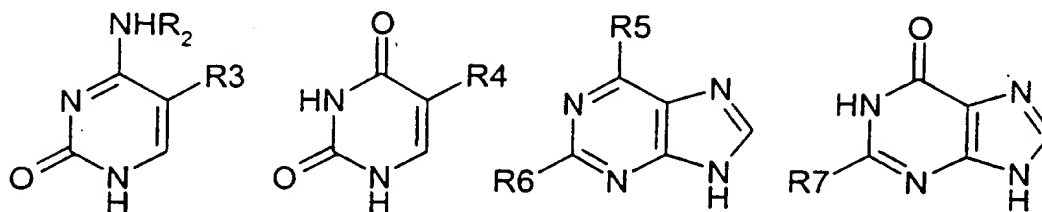
Wherein:

R_2, R_9 and R_{11} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl;

R_3, R_4 and R_{10} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and

R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino.

According to one embodiment, the purine or pyrimidine
base or derivative is selected from the group consisting
of:

In this embodiment, R_2 is selected from the group
consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$
wherein R_8 is hydrogen or C_{1-6} alkyl. Additionally, R_3 and
R_4 are each independently selected from the group
consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine,
fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each
independently selected from the group consisting of
hydrogen, bromine, chlorine, fluorine, iodine, amino,
hydroxyl and C_{3-6} cycloalkylamino.

In another embodiment, the purine or pyrimidine base or
analogue or derivative thereof is selected from the group
consisting of:

In this embodiment, R_9 and R_{11} are independently selected
from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6}
acyl and $R_8C(O)$. Additionally, R_{10} is selected from the

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Next, the compound of formula 3C is deprotected to produce the compound of formula 3D. This can be accomplished by dissolving a compound represented by formula 3C in ethanol and then adding cyclohexene and palladium oxide. The deprotection step can also be done by other methods which are well known by those skilled in the art. The reaction mixture is refluxed for 7 hours. It is then cooled and filtered to remove solids. The solvents are removed from the filtrate by vacuum distillation. The product represented by formula 3D is purified by flash chromatography on silica-gel (5% MEOH in ethylacetate). The deprotection step can also be done by other methods that are well known by a person skilled in the art.

In another embodiment, compounds of Scheme 1 may be prepared by an alternative process which is shown below in Scheme 4.

Scheme 4
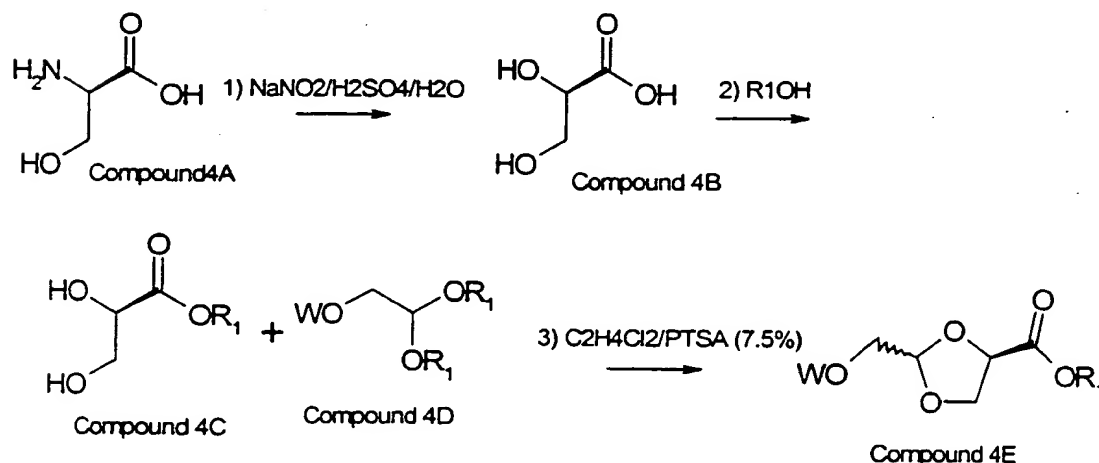
Between about 1.0-1.4 eq of sulfuric acid was added in portions to a large excess of water while stirred at a temperature between 0-5°C. By way of example and not by limitation, if 9.06 mol of D-Serine represents 1

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equivalent of reactant, then between about 9.5-13.3 mol of sulfuric acid is added to 7.3 L of water. In another embodiment, between about 1.1-1.3 eq of sulfuric acid was added to an excess of water. In a further embodiment, 1.2 eq of sulfuric acid was added to an excess of water.

About 1 equivalent of D-Serine was added in one portion under vigorous stirring. Then, between about 1.0 and 1.4 eq. of aqueous sodium nitrite was added dropwise. The temperature was kept between 0-5°C during the addition time (about seven hours). The reaction vessel was stirred overnight at room temperature. The water was removed by vacuum and the residue (D-glyceric acid) co-evaporated with toluene (3X1L). The residue was then stirred with about 6L of an alcohol solvent for about 30 minutes. According to one embodiment, the alcohol is of the formula R_1OH wherein R_1 is a C_{1-4} alkyl. According to another embodiment, the alcohol is methanol or ethanol. The resulting solid was removed by filtration. The clear solution was stirred at room temperature for 30-40 hours, the alcohol removed by vacuum to yield a D-glycerate in the form of a yellow viscous syrup. The D-glycerate is then reacted with between about 0.9-1.1 eq of a dialkyl acetal at a temperature of about 85-95°C. Examples of suitable dialkyl acetals include benzoyloxyacetaldehyde dialkyl acetal and benzyloxyacetaldehyde dialkyl acetal. Examples of suitable alkyls for the dialkyl acetal is methyl and ethyl.

Then, between about 1 wt% and about 10 wt% of PTSA is added. According to another embodiment, about 5 wt% PTSA is added. In another embodiment, about 0.02 eq. of solid PTSA is added. The reaction mixture is kept under vacuum at a temperature between 85-95°C for 2-3 hours. The

mixture is then cooled to room temperature, diluted with
ethylacetate (250 mL) and poured onto saturated sodium
bicarbonate solution (250 mL) under stirring. The
organic phase is separated and the aqueous phase
concentrated, purified on a silica gel column eluting
with 5-10% ethylacetate/hexanes to yield the desired
dioxolane as a light yellow oil (about 59%) with β/α
ratio of 2:1 or higher.

Alternatively, the reactants of step 3 of Scheme 4 can be
substituted by corresponding reactants of Scheme 1. For
example, the D-glycerate represented by Formula 4C is
replaced with an 1,3 dioxolane-4-(4R)carboxylic acid-2,2-
dimethyl alkyl ester represented by Formula 1B according
to one embodiment. Additionally or alternatively, the
dialkyl acetal represented by Formula 4D is replaced with
an oxyaldehyde represented by Formula 1A. These
substitutions do not require changing the reaction
conditions substantially disclosed above for the third
step of Scheme 4.

In a further embodiment of the present invention, the
starting material of Scheme 4 is L-Serine which produces
an end product having an S-configuration at the C4 carbon
of the dioxolane ring. Alternatively, the L-glycerate of
Step 3 can be replaced with an 1,3 dioxolane-4-
(4S)carboxylic acid-2,2-dimethyl alkyl ester to produce
an end product having predominantly an S-configuration at
the C4 carbon of the resulting dioxolane ring.

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Example 1. Enzyme catalyzed hydrolytic resolution of the
dioxolane methyl ester.

A 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-
4-carboxylic acid-1,3-dioxolane methyl ester) (136.5 mg,
0.541mmol) was weighed into a reaction vessel and BES
buffer(6,263 mL of a 5 mM solution, pH 7.2) was added.
The substrate remained as insoluble droplets. α-
Chymotrypsin (500 µL of a 5mg/mL BES buffer, pH 7.2
solution, 0.019 units by 4-nitrophenylacetate assay) was
added to begin the reaction and the rate and degree of
hydrolysis was monitored by a pH-stat which maintained
the pH at 7 by automatic titration with 0.0981 mmol NaOH.
The reaction was terminated at 43% conversion for high
anomeric purity as determined by Sih's equations for
recycling (Chen. C.S.; Fujimoto, Y.; Girdaukas, G.; Sih,
C.J., J.AM. Chem. Soc. 1982, 104, 7294-99), by extracting
the remaining starting material ester with ethyl acetate
(3x20mL). The aqueous layer was adjusted to pH 2 and the
product acid extracted with ethyl acetate (3x20mL).

Both extracts were dried with $MgSO_4$, filtered and
concentrated *in-vacuo*. By this method, we obtained the
(2-(S)-benzyloxymethyl)-4-(S)-carboxylic acid-1,3-
dioxolane methyl ester).

Example 2. Purity of β-Anomer by NMR.

Analysis was performed on a Varian Gemini 200 MHz NMR
spectrometer in $CDCl_3$. The α-ester shows a triplet at δ
5.33 (^3J = 4.6 Hz) and the β-ester shows a triplet upfield
at δ 5.23 (^3J = 4.6 Hz). The α-acid shows a triplet at
δ 5.33(^3J = 3.6 Hz), while the β-acid shows a broad

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singlet upfield at δ 5.19. We did not observe any epimerization of the substrate or product acid during work-up. By NMR analysis, the purity of the β-anomer is determined to have about 95% anomeric purity or higher.

Example 3: Purity of the α-Anomer.

The product acid is obtained from example 1 after it was dried with MgSO₄, filtered and concentrated *in-vacuo*. It is analyzed for purity by NMR. The α-anomer is isolated with high anomeric purity.

Example 4: Enzymatic Resolution of β-anomer with Protease from *Aspergillus oryzae*.

The procedure of Examples 1-2 were followed using protease from Aspergillus oryzae as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 5: Enzymatic Resolution of α-anomer with Protease from *Aspergillus oryzae*.

The product acid is obtained from Example 4 after it is dried with MgSO₄, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

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Example 6: Enzymatic Resolution of β-anomer with Proteinase from *Bacillus licheniformis.*

The procedure of Examples 1-2 were followed using proteinase from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxalane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 7: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus licheniformis.*

The product acid is obtained from Example 6 after it is dried with MgSO$_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 8: Enzymatic Resolution of β-anomer with Subtilisin from *Bacillus licheniformis.*

The procedure of Examples 1-2 were followed using subtilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 9: Enzymatic Resolution of α-anomer with Subtilisin from _Bacillus licheniformis._

The product acid is obtained from Example 8 after it is dried with MgSO₄, filtered and concentrated _in-vacuo_. The α-anomer is isolated with high anomeric purity.

Example 10: Enzymatic Resolution of β-anomer with Protease from _Streptomyces griseus._

The procedure of Examples 1-2 were followed using protease from streptomyces griseus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 11: Enzymatic Resolution of α-anomer with Protease from _Streptomyces griseus._

The product acid is obtained from Example 10 after it is dried with MgSO₄, filtered and concentrated _in-vacuo_. The α-anomer is isolated with high anomeric purity.

Example 12: Enzymatic Resolution of β-anomer with Acylase from _Aspergillus melleus._

The procedure of Examples 1-2 were followed using acylase from aspergillus melleus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

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Example 13: Enzymatic Resolution of α-anomer with Acylase from *Aspergillus melleus*.

The product acid is obtained from Example 12 after it is dried with MgSO₄, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 14: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus subtilis*.

The procedure of Examples 1-2 were followed using proteinase from bacillus subtilis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 15: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus subtilis*.

The product acid is obtained from Example 14 after it is dried with MgSO₄, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 16: Enzymatic Resolution of β-anomer with ESL-001-05

The procedure of Examples 1-2 were followed using diversa clonezymes #5 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic

acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 17: Enzymatic Resolution of α-anomer with ESL-001-05

The product acid is obtained from Example 16 after it is dried with MgSO₄, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 18: Enzymatic Resolution of β-anomer with Pronase protease from *Streptomyces griseus*.

The procedure of Examples 1-2 were followed using pronase from streptomyces as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 19: Enzymatic Resolution of α-anomer with Pronase protease from *Streptomyces griseus*.

The product acid is obtained from Example 18 after it is dried with MgSO₄, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 20: Enzymatic Resolution of β-anomer with Lipase from *Rhizopus arrhizus*.

The procedure of Examples 1-2 were followed using Lipase from rhizopus arrhizus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-

carboxylic acid-1,3-dioxolane methyl ester). The result

is a β-anomer that has high anomeric purity.

Example 21: Enzymatic Resolution of α-anomer with Lipase from *Rhizopus arrhizus*.

The product acid is obtained from Example 20 after it is

dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The

α-anomer is isolated with high anomeric purity.

Example 22: Enzymatic Resolution of β-anomer with Lipoprotein Lipase from Pseudomonas Species Type B.

The procedure of Examples 1-2 were followed using

lipoprotein lipase from pseudomonas sp. type B as the

enzyme to separate a 2:1 (β:α) anomeric mixture of (2-

(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane

methyl ester). The result is a β-anomer that has high

purity.

Example 23: Enzymatic Resolution of α-anomer with Lipoprotein lipase from Pseudomonas Species Type B.

The product acid is obtained from Example 22 after it is

dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The

α-anomer is isolated with high anomeric purity.

Example 24: Enzymatic Resolution of β-anomer with Bacterial Proteinase.

The procedure of Examples 1-2 were followed using

bacterial proteinase as the enzyme to separate a 2:1

- 44 -

(β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-
carboxylic acid-1,3-dioxolane methyl ester). The result
is a β-anomer that has high anomeric purity.

Example 25: Enzymatic Resolution of α-anomer with Bacterial Proteinase.

The product acid is obtained from Example 24 after it is
dried with MgSO$_4$, filtered and concentrated *in-vacuo*. The
α-anomer is isolated with high anomeric purity.

Example 26: Enzymatic Resolution of β-anomer with Lipase from *Pseudomonas cepacia*.

The procedure of Examples 1-2 were followed using lipase
from pseudomonas cepacia as the enzyme to separate a 2:1
(β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-
carboxylic acid-1,3-diaxolane methyl ester). The result
is a β-anomer that has high anomeric purity.

Example 27: Enzymatic Resolution of α-anomer with Lipase from *Pseudomonas cepacia*.

The product acid is obtained from Example 26 after it is
dried with MgSO$_4$, filtered and concentrated *in-vacuo*. The
α-anomer is isolated with high anomeric purity.

Example 28: Enzymatic Resolution of β-anomer with Cholesterol esterase.

The procedure of Examples 1-2 were followed using
cholesterol esterase as the enzyme to separate a 2:1
(β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-

-45-

carboxylic acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 29: Enzymatic Resolution of α-anomer with Cholesterol esterase.

The product acid is obtained from Example 28 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 30: Enzymatic Resolution of β-anomer with ESL-001-02.

The procedure of Examples 1-2 were followed using ESL-001-02 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 31: Enzymatic Resolution of α-anomer with ESL-001-02.

The product acid is obtained from Example 30 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 32: Enzymatic Resolution of β-anomer with Horse Liver Esterase.

The procedure of Examples 1-2 were followed using horse liver esterase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic

-46-

acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 33: Enzymatic Resolution of α-anomer with Horse Liver Esterase.

The product acid is obtained from Example 32 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 34: Enzymatic Resolution of β-anomer with Bovine Pancreatic Protease.

The procedure of Examples 1-2 were followed using bovine pancreatic protease as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 35: Enzymatic Resolution of α-anomer with Bovine Pancreatic Protease.

The product acid is obtained from Example 34 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.


Example 36: Enzymatic Resolution of β-anomer Protease from *Streptomyces caespitosus*.

The procedure of Examples 1-2 were followed using protease from streptomyces caespitosis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-

benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 37: Enzymatic Resolution of α-anomer with Protease from *Streptomyces caespitosus.*

The product acid is obtained from Example 36 after it is dried with MgSO$_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 38: Enzymatic Resolution of β-anomer with Substilisin from *Bacillus licheniformis.*

The procedure of Examples 1-2 were followed using substilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methylester). The result is a β-anomer that has high anomeric purity.

Example 39: Enzymatic Resolution of α-anomer with Substilisin from *Bacillus licheniformis.*

The product acid is obtained from Example 38 after it is dried with MgSO$_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

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<u>Example 40: Preparation of 2-(S)-Benzyloxymethyl-4-(R)-iodo-1,3-dioxolane and 2-(S)-Benzyloxymethyl-4-(S)-iodo-1,3-dioxolane (Compound 40).</u>
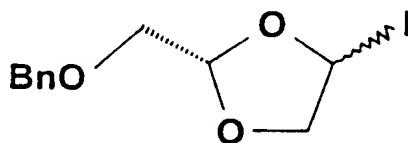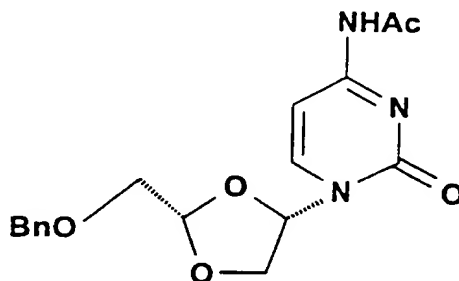

Compound 40

A mixture consisting of 2S-benzyloxymethyl-4S acetoxy-1,3-dioxolane and 2S-benzyloxymethyl-4R-acetoxy-1,3-dioxolane in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic distillation with toluene *in-vacuo*. After removal of toluene, the residual oil was dissolved in dry dichloromethane (60 mL) and iodotrimethylsilane (3.55 mL; 1.05 eq.) was added at -78°C, under vigorous stirring. The dry-ice/acetone bath was removed after addition and the mixture was allowed to warm up to room temperature (15 min.). The product was 2S-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2S-benzyloxymethyl-4S-iodo-1,3-dioxolane.

It would be understood by a person of ordinary skill in the art that if the starting mixture was chosen consisting of 2R-benzyloxymethyl-4S acetoxy-1,3-dioxolane and 2R-benzyloxymethyl-4R-acetoxy-1,3-dioxolane. The resulting product is 2R-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2R-benzyloxymethyl-4S-iodo-1,3-dioxolane. Furthermore, the starting material having a benzoyl substituent group instead of a benzyl would result in a product having a benzoyl substituent and not a benzyl.

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Example 41: Synthesis of 2-(S)-Benzyloxymethyl-1,3-
dioxolan-4-(S)-yl)-2-oxo-4-aminoacetyl-pyrimidine
(Compound 41).

Compound 41

The previously prepared iodo intermediate (Compound 40)
in dichloromethane, was cooled down to -78° C. Persylated
N-acetyl cytosine (1.1 eq) formed by reflux in
1,1,1,3,3,3-hexamethyl disilazane (HMDS) and ammonium
sulphate followed by evaporation of HMDS was dissolved in
30 mL of dichloromethane and was added to the iodo
intermediate. The reaction mixture was maintained at
-78°C for 1.5 hours then poured onto aqueous sodium
bicarbonate and extracted with dichloromethane (2x25mL).
The organic phase was dried over sodium sulphate, the
solid was removed by filtration and the solvent was
evaporated *in-vacuo* to produce 8.1 g of a crude mixture.
β-L-4'-benzyl-2'-deoxy-3'-oxacytidine and its α-L isomer
were formed in a ratio of 5:1 respectively. This crude
mixture was separated by chromatography on silica-gel
(5% methanol in ethylacetate) to generate the pure β-L (β)
isomer (4.48 g). Alternatively, recrystallization of the
mixture from ethanol produces 4.92 g of pure β isomer and
3.18 g of a mixture of β and α-isomers in a ratio of 1:1.

Example 42: 2-(S)-Benzyloxymethyl-1,3-dioxolan-4-(S)-yl)-2-oxo-4-amino-pyrimidine (Compound 42).
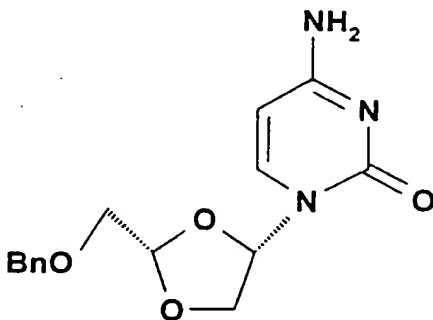
Compound 42

The protected β-L isomer (4.4 g) (Compound 41) was suspended in saturated methanolic ammonia (250 mL) and stirred at room temperature for 18 hours in a closed vessel. The solvents were then removed *in-vacuo* to afford the deacetylated nucleoside in pure form.

Example 43: 2-(S)-hydroxymethyl-1,3-dioxolan-4-(S)-yl)-2-oxo-4-amino-pyrimidine (Compound 43).
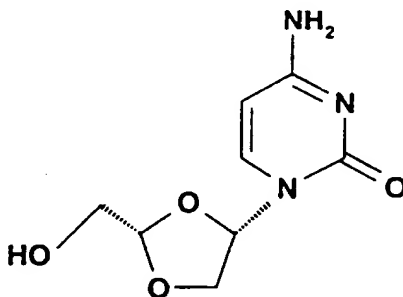
Compound 43

β-L-4'-Benzyl-2'-deoxy-3'-oxacytidine (Compound 42) was dissolved in EtOH (200 mL) followed by addition of cyclohexene (6 mL) and palladium oxide (0.8 g). The reaction mixture was refluxed for 7 hours then it was cooled and filtered to remove solids. The solvents were removed from the filtrate by vacuum distillation. The

crude product was purified by flash chromatography on silica-gel (5% MeOH in EtOAc) to yield a white solid (2.33 g; 86% overall yield). α_D^{22} = -46.7° (c = 0.285; MeOH) m.p. = 192 - 194°C.

The following examples 44-46 illustrate a method of preparing the starting material of example 1 (2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester).

Example 44: Preparation of D-glyceric acid.
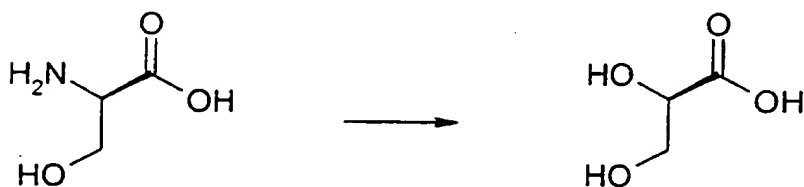
Portions of sulfuric acid (297 mL; 11.14 mol; 1.23 eq) was added to a large excess of water (7,300 mL) under stirring and cooling (0-5°C). D-Serine (952 g; 9.06 mol; 1 eq) was added in one portion under vigorous stirring, followed by dropwise addition of aqueous sodium nitrite (769 g; 11.14 mol; 1.23 eq in 3,060 mL water). Temperature was kept between 0-5°C during the addition time (seven hours). The reaction vessel was stirred overnight at room temperature and the reaction monitored by TLC (ninhydrin). In order to complete the reaction, additional sulfuric acid (115 mL; 4.31 mol; 0.47 eq) and aqueous sodium nitrite (255 g; 3.69 mol; 0.4 eq in 1,100 mL water) was added, keeping the reaction vessel temperature between 0-5°C. The reaction vessel was then kept under stirring at room temperature for another 18 hours. Nitrogen was bubbled through the solution for one hour and the water removed by vacuum, keeping the

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reaction vessel temperature between 28-30°C. The residue
(D-glyceric acid) was co-evaporated with toluene (3X1L).

Example 45: Preparation of D-methyl glycerate.
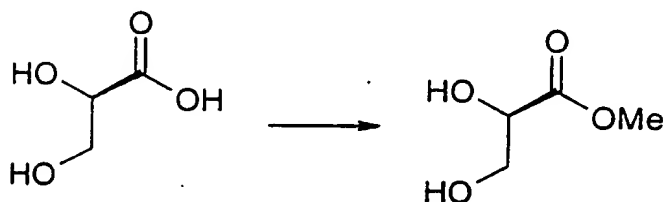
D-glyceric acid was stirred with methanol (6L) for 30
minutes and the solid removed by filtration. The clear
solution was stirred at room temperature for 35-38 hours
and the reaction monitored by TLC (DCM/MeOH 8:2 Rf=0.63).
Methanol was removed by vacuum to yield a yellow viscous
syrup (1,100 g).

Example 46: Preparation of 2-(R,S)-benzoyloxymethyl-4-R-methylcarboxylate-1,3-dioxolane.

A mixture of benzoyloxyacetaldehyde dimethyl acetal (146
g, 95%, 0.66 mole, 1 eq) and D-methyl glycerate (99 g,
0.82 mole, 1.25 eq) was heated to 90oC, followed by the
addition of solid PTSA (2.75 g, 0.145 moles, 0.022 eq).
The reaction mixture was kept under vacuum (water
aspirator) at 90-95°C for 2.5 hours (TLC,
Hexanes/Ethylacetate 1:1, Rf-0.47). The reaction mixture
was cooled down to room temperature, diluted with
ethylacetate (250 mL) and poured onto saturated NaHCO3
solution (250 mL) under stirring. The organic phase was

separated and the aqueous phase was extracted one with
ethylacetate (150 mL). The combined organic phase was
concentrated and purified on a silica gel column eluting
with 5-10% ethylacetate/hexanes to yield 112.4 g of the
desired product as a light yellow oil (59%) with β/α
ratio of 2.1:1.

The following example 47 illustrates a large scale
version of the enzymatic separation of Example 1.

Example 47: Large scale preparation of β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-methylcarboxylate.

A mixture of β/α 2-benzoyloxymethyl-4-methylcarboxylate-
1,3-dioxolane (20 g; 75.12 mmol) was suspended in
acetonitrile (40 mL) and phosphate buffer (160 mL) at
30°C. α-Chymotrypsin was added (7.5 mL) in one portion
followed by dropwise addition of a 1N solution of NaOH
(total volume 46 mL) over six hours to hydrolyze the α
isomer. The pH was constantly monitored and maintained
between 7.1 and 7.2 while the temperature was kept at
30°C. After six hours, the mixture was extracted with
EtOAc (1X80 mL). Then, the organic layer was separated,
and the aqueous layer was extracted with EtOAc (2X50mL).
Combined organic layers were washed with saturated $NaHCO_3$
(20 mL), dried over anhydrous Na_2SO_4 and the solvent
removed under vacuum to leave 12.63 g of a clear oil
(85.6%; β-isomer with less than 2.24% of the α-isomer by
HPLC).

It is understood by a person of ordinary skill in the art
that this example can be followed substituting
chymotrypsin with any of the enzymes used in examples 4
through 39 to result in a large scale production of the
β—isomer of 2-benzoyloxymethyl-4-methylcarboxylate-1,3-
dioxolane.

Example 48. Preparation of β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-carboxylic acid.

β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-
methylcarboxylate-1,3-dioxolane (15.327 g; 57.57 mmol) is
dissolved in THF (60 mL) then water (15 mL) was added
under stirring. The internal temperature was set to 20°C.
Then, a solution of LiOH (2.41 g; 57.57 mmol) in water
(15 mL) was added dropwise over 7 minutes. The reaction
mixture was stirred at 22°C for an additional 40 minutes.
THF was removed under vacuum, and the residue diluted
with water (70 mL). The resulting solution was extracted
with dichloromethane (2X35mL). The aqueous phase was
acidified by 30% H_2SO_4 (9.5 mL) under tight pH-meter
control (initial pH:8.36 to 3.02) then extracted with DCM
(4X60mL). The organic phases were combined and the
solvent removed under vacuum to furnish a light green
syrup (14.26 g) which was kept under vacuum overnight.

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Example 49: Preparation of β 2-(R)-benzoyloxymethyl-4-(R,S)-methylcarboxylate-1,3-dioxolane.
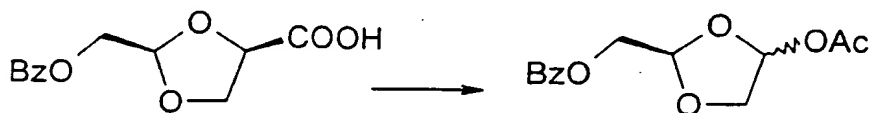
Lead tetraacetate (944, 8 g; 2,024 mole; 1,2 eq) was added portion-wise to an acetonitrile (6.8 L) solution of the acid (425,5 g; 1,687 mole; 1,0 eq) and pyridine (193 mL) in an ice bath. The reaction vessel was allowed to warm up to room temperature and stirred. The reaction was checked by TLC (hexanes:ethyl acetate 6:4). It was filtered through a small pad of celite (about 1 inch). Then, the filtrate was poured onto 5 L of saturated aqueous sodium bicarbonate solution (reaction mixture turned brown), and the pH as adjusted to 8 by adding solid sodium bicarbonate. The filtrate was again filtered through a small pad of celite (about 1 inch) to remove the black lead salts to yield a pale yellow mixture. The organic phase was separated and the aqueous phase was extracted with ethylacetate (4X2L). The combined organic phase was concentrated, and the oil obtained was co-evaporated with toluene (3X2L) to yield a brown syrup.

This syrup (374 g) was further purified by filtering through a small pad of silica gel (1 g crude; 2 g silica), eluting with 3.5 L of the solvent mixture (ethyl acetate:hexanes 8:2) to yield 332,3 g (74%) of pure product. This last filtration step is optional.

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Example 50: Preparation of 9-(2-(R)-benzyloxymethyl-1,3-dioxolan-4-yl)-6-chloro-2-amino purine (Compound 50a) and 9-(2-(R)-benzyloxymethyl-1,3-dioxolan-4-yl)-6-iodo-2-amino purine (Compound 50b).

Compound 50a(b)
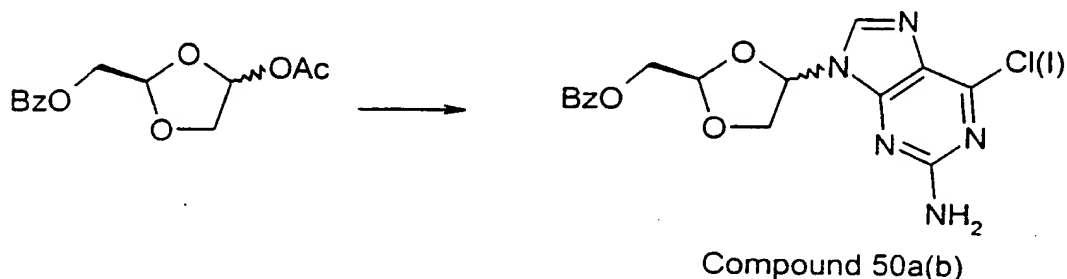
TMSI (28.2 mL; 198.12 mol eq) was added dropwise to a dichloromethane (750 mL) solution of the sugar (2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane)(52.75 g; 198.12 mmol; 1 eq) at -15°C. After 2.5 hr at -15°C, silylated 2-amino-6-chloropurine (62 g; 198 mmol; 1 eq) was added to the reaction mixture as a solid. The stirring was continued at the same temperature for another 2.5 hr. The reaction mixture was allowed to warm up slowly to room temperature followed by continued stirring for 40 hr at room temperature. Then, the mixture was poured onto aq NaHCO₃ solution (1 L). It was stirred for 20 min with Na₂S₂O₃ and filtered through a small pad celite. Then, the organic phase was separated and the aqueous phase was extracted with dichloromethane (1 X 200 mL). The combined organic phases were concentrated to get 87 g of the crude. Column purification of the crude on silica gel (450 g), eluting with ethylacetate/hexane (6:4) yielded 67.7 g (81%; 1:1 chloro/iodo mixture) of the coupled product with β/α ratio 2.3:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the L-configuration).

The procedure discussed above is followed. However, the
sugar 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is
replaced with 2-(S)-benzyloxymethyl-4-carboxyl-1,3-
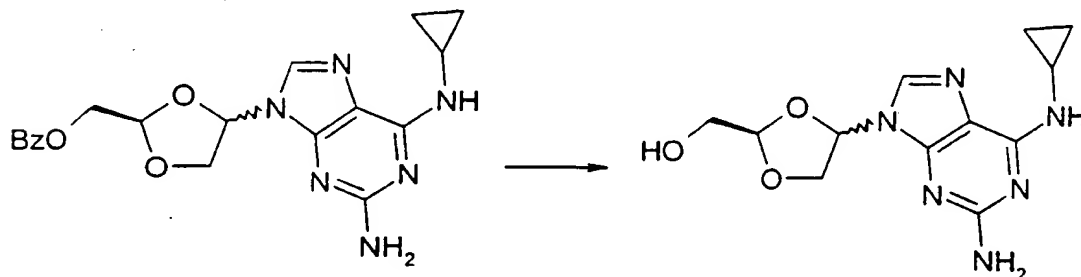dioxolane.

**Example 51: Preparation of 9-(2-(R)-benzyloxymethyl-1,3-
dioxolan-4-yl)-6-(N-cyclopropyl)amino-2-amino purine
Compound 51).**
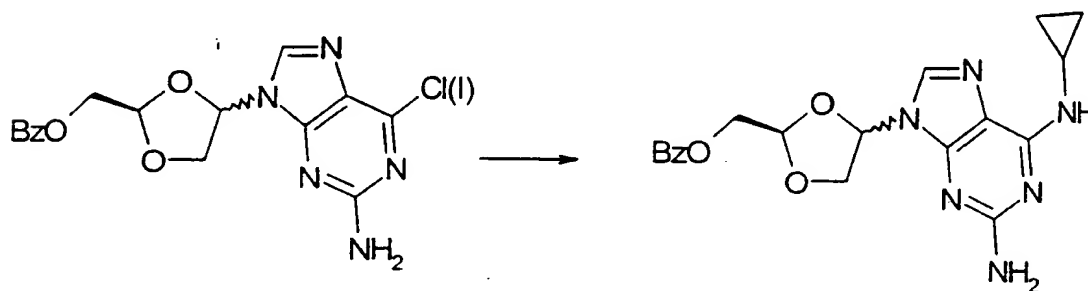
Compound 51

A solution of the starting material (6.3 g; 14.95 mmol; 1
eq; average f.w.=421.52; Cl:I/1:1) in ethanol (100 mL)
was refluxed at 75-80°C with cyclopropylamine (3.1 mL;
44.84 mmol; 3 eq) for 20 hrs and cooled to room
temperature. The reaction mixture was concentrated,
dissolved in dichloromethane (25 mL) and poured onto
saturated aqueous sodium bicarbonate solution. After 10
min. of stirring, the organic phase was separated, and
the aqueous phase was extracted with dichloromethane
(2X15 mL). Then, the combined organic phase was
concentrated to get a quantitative yield of the crude,
which was then purified by column chromatography (silica
gel, ethylacetate:MeOH 98.5:2.5 and 95:5) to yield 5.3 g
(89%) of the product as a β/α mixture.

Example 52: Preparation of 9-(2-(R)-hydroxymethyl-1,3-
dioxolan-4-yl)-6-(N-cyclopropyl)amino-2-amino purine
(Compound 52).

Compound 52

The starting material (3.3 g) was stirred with ammonia in
MeOH (80 mL; 2M) for 20 hrs. Nitrogen was bubbled
through the reaction mixture to remove the excess
ammonia. Then, the solution was concentrated to yield
the crude as a β/α mixture (β/α = 2.3:1). The β/α
isomers were separated by chromatography on silica gel
using DCM/MeOH as eluent to yield 1.18 g (70% β isomer).

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Example 53: Preparation of 9-(2-(R)-hydroxymethyl-1,3-dioxolan-4-yl-6-(N-2-cyclopropyl-2-aminomethoxyl)-2-amino purine (Compound 53).
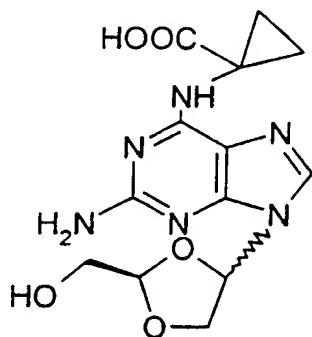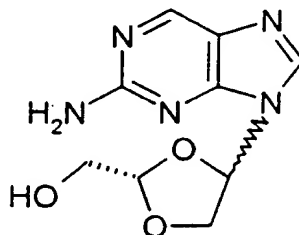

Compound 53

A solution of (2R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane (480 mg) in 30 ml of saturated methanolic ammonia was stirred at room temperature for 18 h. The mixture was evaporated to dryness in vacuo. The residue was dissolved in 20 ml of water, washed twice with 10 ml of methylene chloride and lyophilized to give 283 mg of white solid in 80% yield. The resulting product had a mixture of β:α anomers having a ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the L-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 54: Preparation of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2-amino purine (Compound 54).

Compound 54

The procedure of Example 50 was performed. Thereafter 6.3 g of Compound 50 was subject to hydrogenation conditions under 50 psi of hydrogen over 10% Pd/c in 300 mL of ethanol containing 100 mL of triethylamine. After 3 hours of shaking, the catalyst was removed by filtration. Then the sovent was evaporated to yield a solid which was recrystallised to from ethanol-ether to give about 4 g of Compound 54 having about a 2:1 mixture of β:α stereoisomers in the L-configuration.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. about a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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Example 55: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6-amino purine (Compound 55).
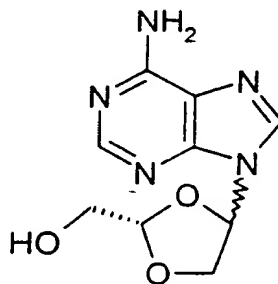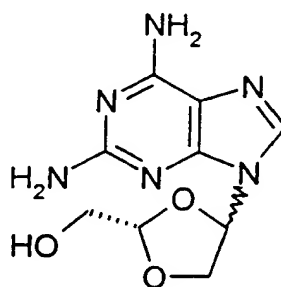

Compound 55

The procedures set forth in Examples 50 and 51 were performed. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 6-aminopurine. The result is a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-6-amino purine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration).
The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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Example 56: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6,2-diamino purine (Compound 56).
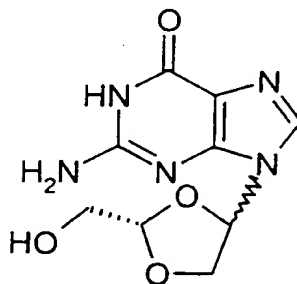

Compound 56

The procedure of Example 50 was performed. Thereafter, 6 g of Compound 50 was dissolved in 0.9 L of methanol saturated at 0°C with dry ammonia and the solution is heated in a steel bomb to 105°C to 110°C for 16 hours. The solution was evaporated to dryness and the residue purified by chromatography on silica gel using chloroform-methanol (4:1) as the eluent to give about 3g of crude Compound 56. The product can be recrystallised from methanol-ether to yield purified Compound 56 having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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Example 57: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6-oxo-2-amino purine (Compound 57).
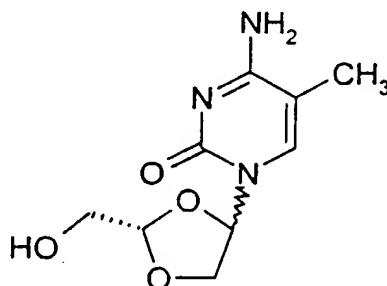
Compound 57

The procedure of Example 50 was performed. Thereafter, about 6 g of Compound 50 was dissolved in a mixture of 200 mL of methanol, 50 mL of water and 10 g of NaoH. The solution was heated under reflux for 5 hours after which time it was diluted with 300 mL of water and excess pyridinium sulfonate resin. The slurry was filtered, the resin washed with water and the combined aqueous filtrates were evaporated to dryness in vacuo to leave a residue which was taken up in 50% aqueous methanol. The solution was treated with activated charcoal, filtered and the filtrate evaporated to dryness in vacuo to give a solic residue that was recrystallized from ethanol water to yield pure compound 57 having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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Example 58: Preparation of 9-(2-(S) hydroxymethyl-1,3-
dioxolan-4-yl)-2-oxo-4-amino-5-methyl pyrimidine
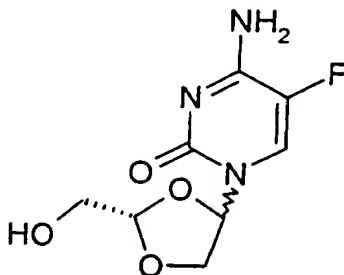(Compound 58).

Compound 58

The procedure of Example 50 was performed followed by the
procedure of Example 52.   However, when following the
steps of Example 50, the 1 equivalent of the silated 2-
amino-6-chloropurine is replaced with 1 equivalent of
silated 2-oxo-4-amino-5-methyl-pyrimidine.   The result is
a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2-
oxo-4-amino-5-methyl pyrimidine having a β:α ratio of
about 2:1.

Alternatively, if the desired final product is the same
compound but with opposite stereochemistry (i.e. a 2:1
mixture of β:α stereoisomers in the D-configuration).
The procedure discussed above is followed. However, when
following the steps of Example 50, the sugar 2-(S)-
benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with
2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 59: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-fluoro pyrimidine (Compound 59).
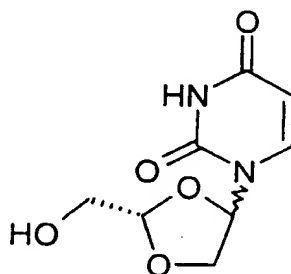

Compound 59

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2-oxo-4-amino-5-fluoro-pyrimidine. The result is a yield of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-fluoro pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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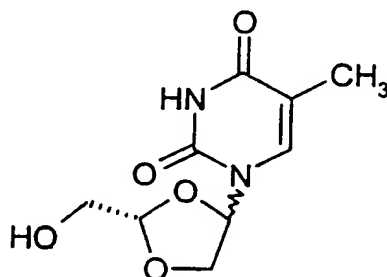Example 60: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo pyrimidine (Compound 60).

Compound 60

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2,4-dioxo pyrimidine. The result is a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration. The above formula is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

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Example 61: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo-5-methyl pyrimidine (Compound 61).

Compound 61

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2,4-dioxo-5-methyl pyrimidine. The result is a yield of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo-5-methyl pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Some modifications and variations of the present invention including but not limited to selection of enzymes with high degree of sequence homology and optimization of reaction conditions will be obvious to a

person of ordinary skill in the art from the foregoing
detailed description of the invention. Such
modifications and variations are intended to fall within
the scope of one or more embodiments of the present
invention as defined by the following claims.

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